ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Glycine Antibodies

Cat # GLYC52-A	Rabbit Anti-Glycine IgG, Aff pure	**SIZE**:100 ug
Cat # GLYC52-N-100	Glycine-BSA protein conjugate for ELISA	**SIZE**:100 ug

Glycine is one of the major inhibitory neurotransmitters in the spinal cord and brain stem. Glycine acts on the strychnine-sensitive glycine receptors, a pentameric Cl-channel, to generate inhibitory postsynaptic potentials. Glycine can modulate excitatory neurotransmission as an obligatory co-agonist with glutamate at NMDA-activated glutamate receptors via binding site on the NMDA receptor, which is distinct from the strychnine-sensitive glycine receptor-binding site. Glycinergic synapses are primarily present throughout the spinal cord, brain stem and cerebellum, as well as in the retina and a few other brain regions. Neurotransmitter action is terminated by re-uptake of glycine via specific high affinity transporter protein located at the plasma membrane of neuron and glial cells.

The glycine transporter belongs to a family of Na/Cl-dependent neurotransmitter transporters, predicted to contain as many as 12 transmembrane domains. Glycine receptor exists in 2 forms - GLYT1 and GLYT2. Form 1 has three other known isoform (GLYT1a, GLYT1b, and GLYT1c) which may be produced by alternative spicing or promoter usage. Rodent Glyt1a and Glyt1b differ only by 10 amino acid at the N-terminus and expressed from the same gene. Although, the N-terminal part of mouse and rat GLYT1a is identical in mouse and rat, the N-terminus of GLYT1b are significantly different in these two species. The GLYT1c subtype has only been reported in humans. GLYT1a is expressed in CNS and peripheral organs. GLYT1b is localized in the CNS. Both neuron and Glial cells have GLYT1. More recent studies indicate that GLYt1b may not be brain specific. Immunolocalization studies on GLYT2 suggest that GLYT2 is responsible for terminating the neurotransmission at the strychnine synapses.

Source of Antigen, Antibodies

Antigen	Glycine was coupled to Keyhole Limpet Hemocyanin (KLH) and injected into rabbits. Resulting antiserum was tested with Glycine-BSA conjugate (cat #GLYC52-N-100) and also affinity purified (Cat # GLYC52-A).
Ab Host/type	Rabbit, Polyclonal IgG, purified over antigen-agarose (Cat # GLYC52-A) supplied in PBS+0.1% BSA+0.05% azide
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
Negative Control IgG	Non-immune rabbit serum IgG (cat # 20009-1) can be used negative control IgG in ELISA, Western or IHC.

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG

100 ug/100ul solution lyophilized powder

Supplied in **Buffer**: PBS+0.1% BSA

Reconstitute powder in PBS at 1mg/ml

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

ELISA (1:1K-10K).

Histochemistry & Immunofluorescence: we recommend 1:100-1:500 using PAP technique and affinity purified antibody at 10-50 ug/ml.

Specificity & Cross-reactivity

Specificity of Anti-Glycine antibodies was tested by an ELISA. No significant crossreaction was observed with beta-alanine, GABA, Taurine, Aspartate, Glutamate-thyroglobulin conjugates or free glycine.

General References:

Schell M et al (1997) J Neuroscience 17, 1604; Spirou G et al (1997) J Comparative Neurol. 383, 473; Gates T et al (1996) Hearing Res. 96, 157.

*This product is for In vitro research use only.

Related material available from ADI

Anti-Rabbit IgG-HRP Conjugate and ECL Reagents

Western Blot Recycling Kit (Strips blots in 5 minutes)

GLYC52-A-N-100

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Rabbit Anti-Glycine Antiserum (cat # GLYC51-S) Recommended protocol for IHC.

SAMPLE PROTOCOL for Neurotransmitter Detection by Immunocytochemistry. Example for a rat brain.

1. **SOLUTIONS TO BE PREPARED** - Solution must be prepared as needed.
Note: Tris can be replaced by a 0.01M phosphate solution.
Solution A: 0.1 M cacodylate acid, 10 g/L sodium metabisulfite, pH 6.2.(*)
Solution B: 0.1 M cacodylate acid, 2.5-5% glutaraldehyde, 10 g/L sodium metabisulfite, pH 7.5.(*)
Solution C: 0.05 M Tris, 8.5 g/L sodium metabisulfite, pH 7.5.(*)
Solution D: 0.05 M Tris, 8.5 g/L sodium chloride pH 7.5.(*)
(*) Adjust pH with NaOH or HCl if necessary.

2. **RAT ANAESTHESIA** - The rat is anaesthetized with sodium pentobarbital or chloral hydrate. The anaesthesia is correct when: on it's back, rat doesn't return to it's side & light reaction occurs pinching the tail.

3. **RAT PERFUSION** - Open the animal's thorax and rapidly cannulate the aorta via the left ventricle. Cut the right atrium or ventricle to allow efflux of blood and perfusate. Clamp off the descending aorta. Perfuse intracardially through the aorta, using either a multi-speed pump or a large syringe.
Solution A (30 mL): 200-300 mL/min
Solution B (500 mL): 200-300 mL/min
Solutions A and B must be perfused through the rat brain continuously without flow stopping when changing solutions.
Indications of a good perfusion:
-Limbs are blanching. Ears are bleached and very white.
-Liver loses it's color and becomes very hard.
-When cutting the rat nose, glutaraldehyde must leak drop by drop.
-The brain must be dark-yellow and hard. (The color is homogeneous without any white blots).
Indications of a incorrect perfusion:
-All the above indications do not appear.
-Glutaraldehyde leaks by the mouth. Rat eyes are swollen.

4. **POST FIXATION:** Cover rat brain with Solution B and let soak 15-30 minutes, then soft wash 4 times in Solution C.
5. **TISSUE SECTIONING:** 50 µm slices, preferably by the "vibratome" technique, using Solution C.

6. **REDUCTION STEP:** Sections are reduced with Solution C containing sodium borohydride (0. 1M) for 10 min. Then the sections are washed carefully 4 times with stock Solution D.
7. **WASHING:** The sections are washed 3X in cold (4 deg) Sol'n C, then incubated 1-1.5 hrs at room temp. in Sol'n C plus 3% of non-specific serum (normal goat serum).
8. **PRIMARY ANTIBODY:** Use a final dilution of 1:1,000-1:2,500 in Solution C containing 0.5% Triton X100 and 2% non-specific serum. Incubate 12 sections per 2 mL diluted antibody overnight, +4°C. Then wash the sections three times for 10 minutes each in Solution D. (Note that the antibody may be usable at a higher dilution. This should be explored to minimize the possibility of high background. Additionally, note that a change in the buffering system as indicated in the protocol may change the background and antibody recognition). The specific reaction is then revealed by PAP procedure.
9. **SECOND ANTIBODY:** Incubate the sections with a 1:50 to 1:200 dilution of goat anti-rabbit in Solution D containing 1% non-specific serum for either 3 hrs at 20°C or 2 hr at 37°C. Then wash the sections, 3 times, for 10 minutes each with Solution D.
10. **PAP:** Incubate the sections with the appropriate dilution of peroxidase anti-peroxidase (for free floating method) in Solution D containing 1% non-specific serum for 1-2 hours at 37°C. Then wash sections 3 times for 10 min each in solution D.
11. **VISUALIZATION:** The antigen-antibody complexes are visualized using DAB-4-HCl (25 mg/100 mL) in 0.05M Tris and filtrated; 0.05% hydrogen peroxide is added. Incubate the sections for 10 minutes at room temp. Stop the reaction by transferring the sections to 5 mL 0.05M Tris. Wash tissue with solution D using 2, 10 min washes. Mount sections on chrome-alum coated slides. Dry overnight at 37°C. Rehydrate sections using conventional histological procedures. Coverslip using rapid mounting